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SYNTHESIS, PHYSICOCHEMICAL AND ANTICONVULSANT PROPERTIES OF NEW N-[(4-ARYLPIPERAZIN-1-YL)ALKYL]-3-PHENYL- AND 3-(3-METHYL-PHENYL)-PYRROLIDINE-2,5-DIONES

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Abstract: The series of N-[(4-arylpiperazin-1-yl)-alkyl]-3-phenyl- and 3-(3-methylphenyl)-pyrrolidine-2,5-diones [**VIII**-**XXV**] were synthesized and evaluated for anticonvulsant and neurotoxic properties. Initial anticonvulsant screening was performed in mice, using intraperitoneal (*ip*) maximal electroshock-induced seizures (MES) and subcutaneous pentylenetetrazole-induced (*sc*PTZ) seizure threshold tests. The neurotoxicity was determined applying the rotorod screen. Compounds **VIII**-**XXV** revealed protection only in the electrically induced seizures or were inactive. The most active were Mannich bases of 3-(3-methylphenyl)-pyrrolidine-2,5-dione with electron-withdrawing substituents at position-3 of 4-arylpiperazine fragment [**XVII**, **XVIII**], as well as compounds with ethylene or propylene spacer between imide and 4-arylpiperazine nitrogen atoms [**XX**-**XXII**, **XXV**]. All these compounds showed anti-MES protection in mice at doses of 100 mg/kg. Additionally, when given orally, compound **XVIII** was also active in rats MES screen at a dose of 30 mg/kg.

Keywords: 3-phenyl-pyrrolidine-2,5-dione, N-[(4-arylpiperazin-1-yl)-alkyl]-3-phenyl-pyrrolidine-2,5-diones; anticonvulsant activity

The nitrogen heterocyclic rings, eg. pyrrolidine-2,5-dione, pyrrolidin-2-one or imidazolidine-2,4-dione are known as one of the important structural fragments appearing in the molecules of currently available anticonvulsants, eg. ethosuximide, phenytoin or levetiracetam. They are also core fragments of compounds obtained during studies on the search of new antiepileptic drugs (1-4). Therefore in the course of developing new anticonvulsants, in recent years we have synthesized many of pyrrolidine-2,5-diones variously substituted at the nitrogen atom as well as at the position-3 of the imide ring (5-11). In the earlier studies, we have reported the synthesis and anticonvulsant activity of N-[(4-arylpiperazin-1-yl)-alkyl]-3-phenyl-pyrrolidine-2,5-diones which displayed potent anti-seizure protection in the maximal electroshock test (12, 13). Among these, the most active were compounds with methylene bridge between two nitrogen atoms and (2-chlorophenyl)- [**I**], (2-trifluoromethylphenyl)- [**II**] and (2-methylphenyl)- [**III**] moieties at position-3 of the imide ring (Fig. 1).

The structure-activity relationship analysis indicated that anticonvulsant activity was connected closely with the presence of electron-withdrawing substituents at the 3-phenyl ring. As a continuation of our systematic SAR studies in this group of derivatives, in the present work we have designed and synthesized a new series of N-[(4-arylpiperazin-1-yl)-methyl]-3-phenyl-pyrrolidine-2,5-diones [**VIII-XIII**] and their analogues with the methyl group at position-3 of the 3-phenyl ring [**XIV-XIX**]. Additionally, we have obtained several derivatives with ethylene or propylene spacer between piperazine and imide nitrogen atoms [**XX-XV**].

Compounds **VIII**-**XXV** were synthesized according to Scheme 1. The starting materials, 2-phenyl- (**IV**) and 2-(3-methylphenyl)-(**V**) succinic acids were prepared using the methods reported elsewhere (14, 15). The 3-phenyl- and 3-(3-methylphenyl)-pyrrolidine-2,5-diones (**VI**, **VII**) were obtained in the cyclization reaction (ca. 190^{o}C, 2 h) of the dicarboxylic acids **IV** or **V** with 25% ammonia. Compounds **VIII**-**XIX** were synthesized by the use of Mannich-type reaction from the appropriately substituted 3-phenylpyrrolidine-2,5-diones (**VI**, **VII**), formaldehyde and the corresponding 4-arylpiperazines. The reaction was performed in

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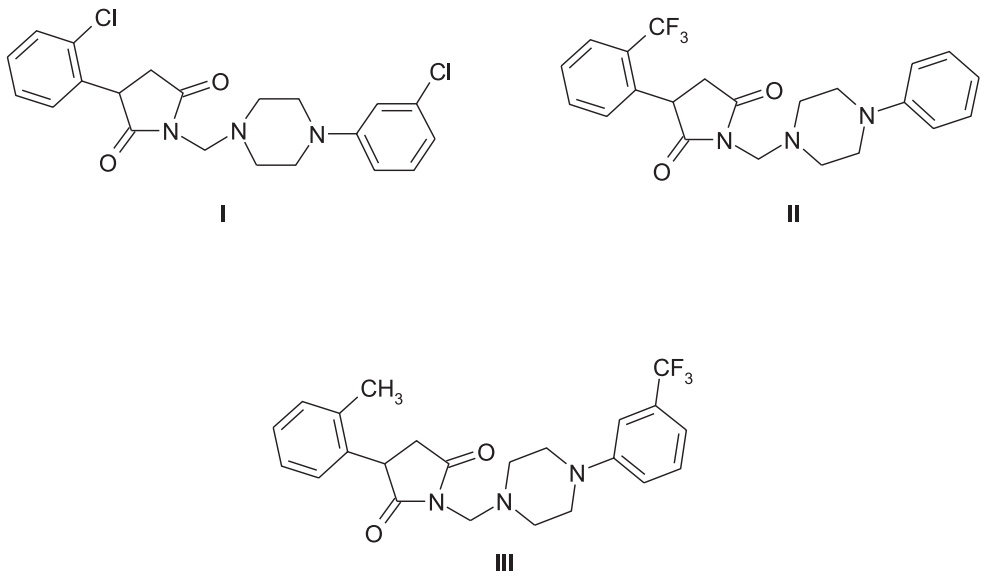
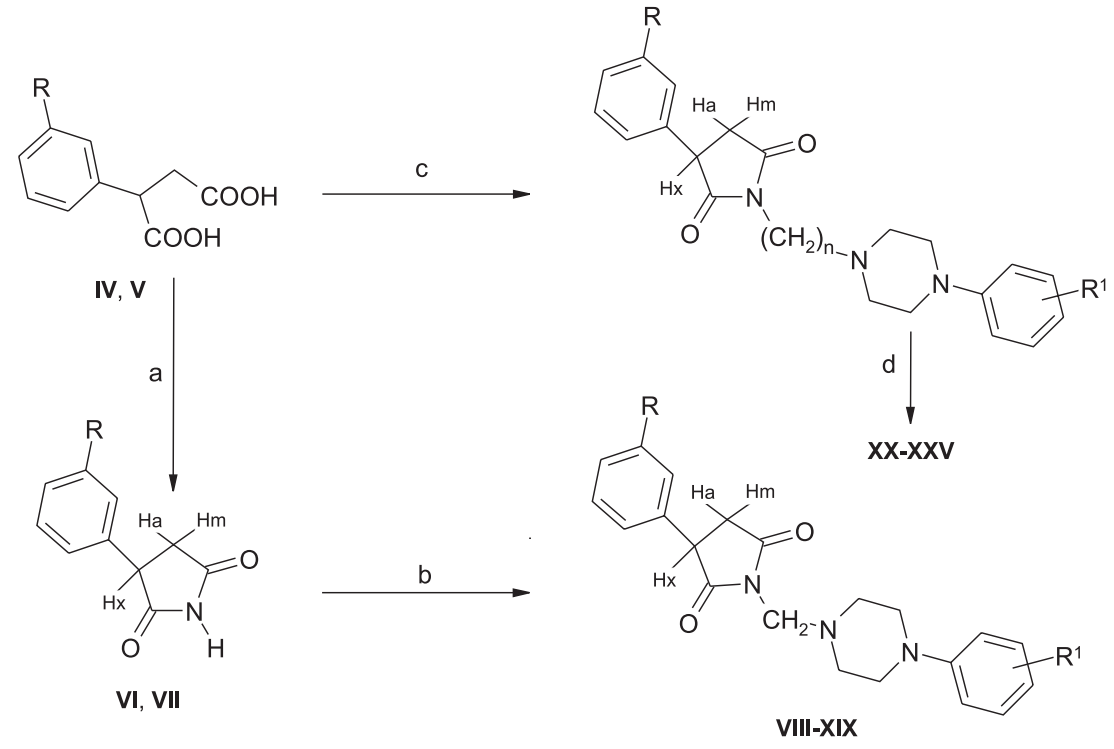


Figure 1. Chemical structures of compounds **I-III**.



R = H, CH_3; n = 2, 3; R^1 = H, 2-F, 3-Cl, 4-Cl, 3-CF_3, 3-CH_3

Scheme 1. Synthesis of compounds **VI-XXV**.
Reagents and conditions: (a) 25% NH_4OH, 190°C, 2 h; (b) 4-arylpiperazine derivatives, formaldehyde, 96% ethyl alcohol, reflux for 0.5 h or ca. 6-12 h room temp; (c) 1-aminoalkyl-4-arylpiperazine, cyclocondensation, 190°C, 2 h; (d) anhydrous ethanol + HCl

Table 1. Physicochemical and analytical data for compounds **VI**-**XXV**.

Compd.	Molecular formula/Weight	Yield % m.p. [°C]	Analysis			R_f[a]
			%C	%H	%N	
VI	$C_{10}H_9O_2N$ 175.19	70 78-80	68.64 68.90	5.18 5.27	8.00 7.83	0.42 (S_1)
VII	$C_{11}H_{11}O_2N$ 189.17	60 91-93	69.91 70.03	5.87 6.00	7.41 7.66	0.53 (S_1)
VIII	$C_{21}H_{23}O_2N_3$ 349.44	72 154-156	72.17 72.16	6.63 6.71	12.03 12.12	0.70 (S_2)
IX	$C_{21}H_{22}O_2N_3F$ 367.43	81 93-94	68.65 68.55	6.04 6.24	11.44 11.23	0.65 (S_2)
X	$C_{21}H_{22}O_2N_3Cl$ 383.88	73 102-104	65.70 65.75	5.78 5.81	10.95 11.01	0.66 (S_2)
XI	$C_{21}H_{22}O_2N_3Cl$ 383.88	73 114-116	65.70 65.78	5.78 5.77	10.95 10.90	0.63 (S_2)
XII	$C_{22}H_{22}O_2N_3F_3$ 417.44	79 116-117	63.30 63.18	5.31 5.42	10.07 10.20	0.69 (S_2)
XIII	$C_{22}H_{25}O_2N_3$ 363.46	84 102-103	72.69 72.35	6.93 6.97	11.56 11.44	0.72 (S_2)
XIV	$C_{22}H_{25}O_2N_3$ 363.46	69 82-84	72.69 72.62	6.93 6.87	11.56 11.62	0.61 (S_2)
XV	$C_{22}H_{24}O_2N_3F$ 381.45	83 118-120	69.27 69.17	6.34 6.13	11.02 11.00	0.63 (S_2)
XVI	$C_{22}H_{24}O_2N_3Cl$ 397.90	82 85-87	66.41 66.35	6.08 6.27	10.56 10.53	0.69 (S_2)
XVII	$C_{22}H_{24}O_2N_3Cl$ 397.90	63 132-134	66.39 66.34	6.08 6.01	10.56 10.55	0.72 (S_2)
XVIII	$C_{23}H_{24}O_2N_3F_3$ 431.46	75 80-82	64.03 63.95	5.61 5.70	9.74 9.66	0.94 (S_2)
XIX	$C_{23}H_{27}O_2N_3$ 377.49	65 91-93	73.18 73.11	7.21 7.11	11.13 11.12	0.68 (S_2)
XX	$C_{23}H_{25}O_2N_3ClF_3$ 467.92	70 210-212	59.04 58.98	5.39 5.62	8.98 8.80	0.79 (S_2)
XXI*	$C_{23}H_{29}O_2N_3Cl_2$ 450.41	58 191-193	61.33 61.30	6.49 6.38	9.33 9.21	0.79 (S_2)
XXII	$C_{23}H_{28}O_2N_3Cl$ 413.95	65 190-192	66.74 66.92	6.82 7.02	10.15 9.95	0.91 (S_2)
XXIII	$C_{23}H_{27}O_2N_3ClF$ 431.94	65 179-181	63.96 63.78	6.30 6.18	9.73 9.55	0.80 (S_2)
XXIV	$C_{23}H_{27}O_2N_3Cl$ 190.20	62 178-180	61.61 61.45	6.07 6.00	9.37 9.21	0.85 (S_2)
XXV	$C_{24}H_{29}O_2N_3Cl_2$ 462.43	68 180-182	62.40 62.62	6.33 6.17	9.10 9.30	0.82 (S_2)

[a] Developing system: S_1 – chloroform: acetone (9 : 1, v/v), S_2 – chloroform : 2-propanol : 25% ammonia (9 : 11 : 2, v/v/v).
* Physicochemical data from Ref. (17)

ethanol at room temperature for ca. 6-12 h. Additionally, in several cases the reaction mixture was refluxed for 0.5 h.

Compounds **XX**-**XXV** were prepared using a one-pot cyclization reaction of **IV** or **V** with appropriately substituted 1-(2-aminoethyl)- or 1-(3-aminopropyl)-4-arylpiperazines, by heating them at ca. 190°C for 2 h. Because of the oily form of compounds **XX**-**XXV** they were isolated as hydrochloride salts.

The 1-(2-aminoethyl)- and 1-(3-aminopropyl)-4-arylpiperazines were prepared according to the

Table 2. ^{1}H-NMR data of compounds **VI**-**XIII**.

Cmpd	^{1}H NMR δ (ppm) / $CDCl_3$
VI	2.90 (dd, 1H, H_a imide, *J* = 5.13 Hz, *J* = 18.72 Hz), 3.26 (dd, 1H, H_m imide, *J* = 9.50 Hz, *J* = 18.46 Hz), 4.10 (q, 1H, H_x imide, *J* = 5.13 Hz), 7.27-7.42 (m, 5H, ArH)
VII	2.35 (s, 3H, CH_3), 2.87 (dd, 1H, H_a imide, *J* = 5.13 Hz, *J* = 18.72 Hz), 3.24 (dd, 1H, H_m imide, *J* = 9.75 Hz, *J* = 18.72 Hz), 4.04 (q, 1H, H_x imide, *J* = 5.13 Hz), 7.27-7.42 (m, 5H, ArH), 7.03-7.05 (m, 2H, ArH), 7.15 (t, 1H, ArH, *J* = 7.45 Hz), 7.27-7.30 (m, 1H, ArH)
VIII	2.91 (d, 1H, H_a imide, *J* = 5.13 Hz), 2.77-2.86 (m, 4H, piperazine), 3.17-3.17 (m, 4H, piperazine), 3.23 (dd, 1H, H_m imide, *J* = 9.75 Hz, *J* = 18.50 Hz), 4.05 (q, 1H, H_x imide, *J* = 4.62 Hz), 4.61 (t, 2H, $\underline{CH_2}$, *J* = 13.59 Hz), 6.84-6.92 (m, 3H, ArH), 7.22-7.41 (m, 7H, ArH)
IX	2.81-2.87 (m, 4H, piperazine), 2.92 (d, 1H, H_a imide, *J* = 5.13 Hz), 3.06-3.13 (m, 4H, piperazine), 3.25 (dd, 1H, H_m imide, *J* = 9.74 Hz, *J* = 18.46 Hz), 4.07 (q, 1H, H_x imide, *J* = 5.13 Hz), 4.60 (t, 2H, $\underline{CH_2}$, *J* = 13.33 Hz), 6.90-7.08 (m, 4H, ArH), 7.24-7.42 (m, 5H, ArH)
X	2.77-2.83 (m, 4H, piperazine), 2.90 (dd, 1H, H_a imide, *J* = 4.87 Hz, *J* = 18.72 Hz), 3.15-3.19 (m, 4H, piperazine), 3.24 (dd, 1H, H_m imide, *J* = 9.49 Hz, *J* = 18.46 Hz), 4.06 (q, 1H, H_x imide, *J* = 5.0 Hz), 4.60 (t, 2H, $\underline{CH_2}$, *J* = 13.59 Hz), 6.74-6.85 (m, 3H, ArH), 7.16 (t, 1H, ArH, *J* = 8.08 Hz), 7.22-7.41 (m, 5H, ArH)
XI	2.76-2.79 (m, 4H, piperazine), 2.89 (dd, 1H, H_a imide, *J* = 5.13 Hz, *J* = 18.46 Hz), 3.12-3.15 (m, 4H, piperazine), 3.24 (dd, 1H, H_m imide, *J* = 9.74 Hz, *J* = 18.72 Hz), 4.05 (q, 1H, H_x imide, *J* = 5.13 Hz), 4.60 (t, 2H, $\underline{CH_2}$, *J* = 13.34 Hz), 6.80-6.83 (m, 2H, ArH), 7.17-7.22 (m, 4H, ArH), 7.28-7.40 (m, 3H, ArH)
XII	2.79-2.81 (m, 4H, piperazine), 2.89 (dd, 1H, H_a imide, *J* = 5.13 Hz, *J* = 18.98 Hz), 3.20 (brs, 4H, piperazine), 3.27 (dd, 1H, H_m imide, *J* = 9.74 Hz, *J* = 18.46 Hz), 4.06 (q, 1H, H_x imide, *J* = 5.13 Hz), 4.61 (t, 2H, $\underline{CH_2}$, *J* = 13.34 Hz), 7.02-7.09 (m, 3H, ArH), 7.22-7.26 (m, 2H, ArH), 7.31-7.40 (m, 4H, ArH)
XIII	2.31 (s, 3H, $\underline{CH_3}$), 2.79 (brs, 4H, piperazine), 2.88 (dd, 1H, H_a imide, *J* = 5.13 Hz, *J* = 18.46 Hz), 3.14-3.18 (m, 4H, piperazine), 3.24 (dd, 1H, H_m imide, *J* = 9.74 Hz, *J* = 18.46 Hz), 4.06 (q, 1H, H_x imide, *J* = 5.00 Hz), 4.60 (s, 2H, $\underline{CH_2}$), 6.61-6.75 (m, 3H, ArH), 7.13-7.17 (m, 1H, ArH), 7.22-7.26 (m, 2H, ArH), 7.31-7.40 (m, 3H, ArH)

procedure described elsewhere (16). The synthesis and physicochemical data of **XXI** have been described previously (17). This molecule was not tested for its anticonvulsant activity

The structures of **VI**-**XXV** were confirmed by the elemental analysis and by the examination of ^{1}H NMR spectra.

The ^{1}H NMR spectra revealed characteristic chemical shifts. The protons of imide rings (H_a, H_m, H_x) were separated and revealed the known AMX system. The signals of H_a protons were observed as doublet of doublets within the range from δ 2.67 to 3.02 ppm [**VI**, **VII**, **X**-**XIV**, **XVI**, **XVIII**, **XX**-**XXV**] or as doublets at about δ 2.90 ppm [**XVII**, **VIII**, **XV**, and **XIX**]. The H_m protons were recorded as doublet of doublets within the range δ 3.23 to 3.32 ppm [**VI**-**XV** and **XXV**], as doublets at δ 3.25 [**XVI**, **XVII**] and at δ 3.26 ppm [**XIX**] or as multiplets within the range δ 3.38-3.74 ppm [**XVIII**, **XX**-**XXIV**]. For all compounds the resonance signals of H_x protons were shown as quartets ranging from δ 4.01 to 4.82 ppm. The protons of methylene group [**VIII**-**XII**, **XIV** and **XV**] appeared as triplets at δ 4.60 ppm or as singlets for compounds **XIII** and **XIV**-**XIX**. The piperazine protons were observed as multiplets or broad singlets at about δ 2.80 ppm and δ 3.70 ppm. The resonance signals of methyl groups [**XV**-**XIX** and **XXII**-**XV**] were shown as singlets ranging from δ 2.34 ppm to δ 2.40 ppm. All aromatic protons were well separated and were observed within a range of δ 6.60-7.42 ppm. For the details see Tables 2, 3 and 4.

EXPERIMENTAL

Chemistry

All the chemicals and solvents were purchased from Merck (Darmstadt, Germany) and were used without further purification. Melting points (m.p.) were determined in open capillaries on a Büchi 353 melting point apparatus (Büchi Labortechnik, Flawil, Switzerland) and are uncorrected. The purity of the compounds was confirmed by the thin-layer chromatography (TLC) on Merck silica gel 60 F_{254} aluminium sheets (Merck; Darmstadt, Germany), using the developing systems: S_1 – chloroform : acetone (9 : 1, v/v). S_2 – chloroform : 2-propanol : 25% ammonia (9 : 11 : 2, v/v/v). Spots were detected by their absorption under UV light (λ = 254 nm) and by visualization with 0.05 mol I_2 in 10% HCl. The chemical structures were confirmed by elemental

Table 3. ^{1}H-NMR data of compounds **XIV**-**XX**.

Compd.	^{1}H NMR δ (ppm) / $CDCl_3$
XIV	2.43 (s, 3H, $\underline{CH_3}$), 2.80-2.82 (m, 4H, piperazine), 2.88 (dd, 1H, H_a imide, *J* = 4.87 Hz, *J* = 18.46 Hz), 3.16-3.20 (m, 4H, piperazine), 3.23 (dd, 1H, H_m imide, *J* = 9.74 Hz, *J* = 18.72 Hz), 4.01 (q, 1H, H_x imide, *J* = 5.00 Hz), 4.60 (t, 2H, $\underline{CH_2}$, *J* = 13.08 Hz), 6.85-6.93 (m, 3H, ArH), 7.01-7.05 (m, 2H, ArH), 7.11-7.24 (m, 1H, ArH), 7.27-7.30 (m, 3H, ArH)
XV	2.35 (s, 3H, $\underline{CH_3}$), 2.85 (brs, 4H, piperazine), 2.91 (d, 1H, H_a imide, *J* = 87 Hz), 3.07-3.10 (m, 4H, piperazine), 3.23 (dd, 1H, H_m imide, *J* = 9.74 Hz, *J* = 18.46 Hz), 4.03 (q, 1H, H_x imide, *J* = 4.87 Hz), 4.61 (t, 2H, $\underline{CH_2}$, *J* = 13.08 Hz), 6.90-6.99 (m, 2H, ArH), 7.02-7.08 (m, 3H, ArH), 7.11-7.29 (m, 3H, ArH)
XVI	2.34 (s, 3H, CH_3), 2.75-2.78 (m, 4H, piperazine), 2.87 (dd, 1H, H_a imide, *J* = 4.87 Hz, *J* = 18.72 Hz), 3.15-3.20 (m, 4H, piperazine), 3.25 (d, 1H, H_m imide, *J* = 9.75 Hz), 4.11 (q, 1H, H_x imide, *J* = 4.87 Hz), 4.59 (s, 2H, $\underline{CH_2}$), 6.73-6.85 (m, 3H, ArH), 7.00-7.28 (m, 5H, ArH)
XVII	2.34 (s, 3H, CH_3), 2.78-2.85 (m, 4H, piperazine), 2.90 (d, 1H, H_a imide, *J* = 4.87 Hz), 3.12-3.20 (m, 4H, piperazine), 3.25 (d, 1H, H_m imide, *J* = 9.49 Hz), 4.01 (q, 1H, H_x imide, *J* = 4.87 Hz), 4.59 (s, 2H, $\underline{CH_2}$), 6.81 (d, 2H, ArH, *J* = 8.98 Hz), 7.00-7.04 (m, 2H, ArH), 7.11-7.28 (m, 4H, ArH)
XVIII	2.35 (s, 3H, CH_3), 3.02 (dd, 1H, H_a imide, *J* = 5.13 Hz, *J* = 18.72 Hz), 3.38-3.47 (m, 5H, 4H piperazine, 1H, H_m imide), 3.70 (brs, 4H, piperazine), 4.25 (q, 1H, H_x imide, *J* = 5.64 Hz), 4.82 (s, 2H, $\underline{CH_2}$), 7.06-7.30 (m, 7H, ArH), 7.43 (t, 1H, ArH, *J* = 7.95 Hz)
XIX	2.33 (d, 6H, 2xCH_3, *J* = 8.21 Hz), 2.84 (brs, 4H piperazine), 2.91 (d, 1H, H_a imide, *J* = 4.87 Hz), 3.17-3.22 (m, 4H, piperazine), 3.26 (d, 1H, H_m imide, *J* = 9.49 Hz), 4.02 (q, 1H, H_x imide, *J* = 4.87 Hz), 4.60 (s, 2H, $\underline{CH_2}$), 6.65-6.85 (m, 3H, ArH), 7.03 (d, 2H, ArH, *J* = 8.97 Hz), 7.14 (t, 2H, ArH, *J* = 7.58 Hz), 7.24-7.30 (m, 1H, ArH)
XX	2.83 (dd, 1H, H_a imide, *J* = 4.62 Hz, *J* = 18.46 Hz), 2.93-2.96 (m, 2H, CH_2-$\underline{CH_2}$), 3.36-3.38 (m, 2H, piperazine), 3.61-3.67 (m, 3H, 1H, H_m imide, 2H, $\underline{CH_2}$-CH_2), 3.70-3.80 (m, 2H, piperazine), 3.93-4.05 (m, 4H, piperazine), 4.57 (q, 1H, H_x imide, *J* = 4.87 Hz), 7.06-7.60 (m, 9H, ArH), 12.51 (brs, 1H, HCl)

and spectral analyses (^{1}H NMR). ^{1}H NMR spectra were obtained in a Varian Mercury 300 MHz spectrometer (Varian Inc., Palo Alto, CA, USA), in $CDCl_3$, with TMS as an internal standard. Chemical shifts are reported in δ values (ppm) and *J* values in Hertz (Hz). Signal multiplicities are represented by the following abbreviations: s (singlet), brs (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet). Elemental analyses C, H, N were carried out with an Elementar Vario EL III (Hanau, Germany) and were within ± 0.4% of the theoretical values. The physicochemical data, yields, elemental analyses and R_f values for compounds **VI**-**XXV** are presented in Table 1. The ^{1}H NMR data are shown in Tables 2-4.

General procedure for the synthesis of 3-phenyl- and 3-(methylphenyl)-pyrrolidine-2,5-diones (**VI, VII**)

A total of 0.05 mol of the 2-phenyl- (**IV**) or 2-(3-methylphenyl)- (**V**) succinic acids were dissolved in 50 mL of water and 0.05 mol of a 25% ammonia was gradually added. The mixture was heated in an oil bath with simultaneous distillation of water. The cyclization reaction was continued at 190°C for 2 h. The solid products were separated by filtration and crystallized from methanol.

General procedure for the synthesis of N-[(4-arylpiperazin-1-yl)-methyl]-3-phenyl- (VIII-XIII) and 3-(3-methylphenyl)-pyrrolidine-2,5-diones **(XIV-XIX)**

The mixture of 3-phenyl- (0.01 mol) (**VI**) or 3-(3-methylphenyl)-pyrrolidine-2,5-dione (0.01 mol) (**VII**), 40% formaldehyde (0.01 mol) and corresponding 4-arylpiperazines (0.01 mol) in 96% ethanol (40 mL) was left for 6-12 h at room temperature (additionally refluxed for compounds **XIV** and **XX**), then refrigerated at ca. -10°C for 24 h. The solid products (**VIII-XIII**, and **XIV-XIX**) were separated by filtration and recrystallized from 96% ethanol.

General procedure for the synthesis of N-[(4-arylpiperazin-1-yl)-alkyl]-3-phenyl- (XX, XXI) and 3-(3-methylphenyl)-pyrrolidine-2,5-diones **(XII-XXV)**

A total of 0.01 mol of the appropriately substituted 1-(2-aminoethyl)- or 1-(3-aminopropyl)-4-arylpiperazines were mixed with 20 mL of water, and 0.01 mol of the 2-phenyl- (**IV**) or 2-(3-methylphenyl)- (**V**) succinic acid was gradually added. The mixture was heated in an oil bath with simultaneous distillation of water. The cyclization reaction was continued in 190°C for 2 h. Free bases,

Table 4. ^{1}H-NMR data of compounds **XXI**-**XXV**.

Cmpd	^{1}H NMR δ (ppm) / $CDCl_3$
XXI*	2.06 (t, 2H, CH_2-$\underline{CH_2}$-CH_2, *J* = 7.60 Hz), 2.67 (dd, 1H, H_a imide, *J* = 4.87 Hz, *J* =18.20 Hz), 2.99 (brs, 2H, -CH_2-CH_2-$\underline{CH_2}$), 3.36 (d, 2H, $\underline{CH_2}$-CH_2-CH_2, *J* = 5.39 Hz), 3.60-3.74 (m, 5H, 4H piperazine, 1H, H_m imide), 3.95-4.06 (m, 4H, piperazine), 4.82 (q, 1H, H_x imide, *J* = 4.62 Hz), 6.93-7.32 (m, 9H, ArH), 12.72 (brs, 1H, HCl)
XXII	2.34 (s, 3H, CH_3), 2.71 (dd, 1H, H_a imide, *J* = 4.67 Hz, *J* = 18.43 Hz), 2.96 (brs, 2H, CH_2-$\underline{CH_2}$), 3.32-3.59 (m, 2H, $\underline{CH_2}$-CH_2), 3.62-3.69 (m, 5H, 4H piperazine, 1H, H_m imide), 3.98 (brs, 4H, piperazine), 4.52 (q, 1H, H_x imide, *J* = 4.67 Hz), 6.92-6.99 (m, 2H, ArH), 7.04-7.21 (m, 2H, ArH), 7.22-7.32 (m, 5H, ArH), 12.70 (brs, 1H, HCl)
XXIII	2.34 (s, 3H, CH_3), 2.80 (dd, 1H, H_a imide, *J* = 4.36 Hz, *J* = 18.21 Hz), 3.00 (d, 2H, CH_2-$\underline{CH_2}$, *J* = 8.98 Hz), 3.36-3.59 (m, 4H, piperazine), 3.60-3.73 (m, 3H, 2H, $\underline{CH_2}$-CH_2, 1H, H_m imide), 3.98 (brs, 4H, piperazine), 4.53 (q, 1H, H_x imide, *J* = 4.36 Hz), 6.95-7.11 (m, 6H, ArH), 7.21-7.26 (m, 2H, ArH), 12.44 (brs, 1H, HCl)
XXIV	2.40 (s, 3H, CH_3), 2.68 (dd, 1H, H_a imide, *J* = 4.62 Hz, *J* = 18.20 Hz), 2.93 (d, 2H, CH_2-$\underline{CH_2}$, *J* = 8.72 Hz), 3.35-3.40 (m, 2H, $\underline{CH_2}$-CH_2,), 3.60-3.95 (m, 5H, 1H, H_m imide, 4H, piperazine), 3.98-4.07 (m, 4H, piperazine), 4.82 (q, 1H, H_x imide, *J* = 4.87 Hz), 7.04-7.23 (m, 7H, ArH), 7.39 (t, 1H, ArH, *J* = 7.95 Hz), 12.88 (brs, 1H, HCl)
XXV	1.61 (brs, 2H, (m, 2H, CH_2-$\underline{CH_2}$-CH_2), 2.33-2.36 (m, 4H, piperazine), 2.39 (s, 3H, CH_3), 2,89 (dd, 1H, H_a imide, *J* = 4.68 Hz, *J* = 18.40 Hz), 3.08 (brs, 2H, CH_2-CH_2-$\underline{CH_2}$), 3.32 (dd, 1H, H_m imide, *J* = 9.63 Hz, *J* = 18.70 Hz), 3.65 (brs, 4H, piperazine), 3.75 (t, 2H, $\underline{CH_2}$-CH_2-CH_2, *J* = 6.33 Hz), 4.12 (q, 1H, H_x imide, *J* = 4.68 Hz), 6.79-6.82 (m, 1H, ArH), 6.92 (t, 1H, ArH, *J*= 2.20 Hz), 6.95-7.08 (m, 2H, ArH), 7.14-7.32 (m, 4H, ArH), 12.80 (brs, 1H, HCl) * Data from Ref. (17)

obtained as light oils, were converted to hydrochloride salts in anhydrous ethanol saturated with HCl gas. The obtained precipitates were crystallized from anhydrous ethanol.

Pharmacology

Compounds **VIII**-**XXV** were pharmacologically pre-evaluated within the Antiepileptic Drug Development (ADD) Program in Epilepsy Branch, National Institutes of Health, National Institute of Neurological Disorders and Stroke (NIH/NINDS), Rockville, MD, USA, by using procedures described elsewhere (18).

Male albino mice (CF–1 strain) and male albino rats (Sprague–Dawley) were used as experimental animals. The animals were housed in metabolic cages and allowed free access to food and water. The compounds were suspended in 0.5% methylcellulose/water mixture.

Phase I studies in mice involved two convulsant testes: maximal electroshock seizure test (MES), subcutaneous pentylenetetrazole seizure test (*sc*PTZ) and rotorod test for neurological toxicity (NT).

The maximal electroshock test (MES)

In the MES screen, an electrical stimulus of 0.2 s in duration (50 mA in mice and 150 mA in rat at 60 Hz) is delivered *via* corneal electrodes primed with an electrolyte solution containing an anesthetic agent.

The subcutaneous pentylenetetrazole seizure test (*sc*PTZ)

This screen utilizes a dose of pentylenetetrazole (85 mg/kg in mice and 70 mg/kg in rats) that produces clonic seizures lasting for a period of at least five seconds in 97% (CD_{97}) of animals tested. At the anticipated time of testing the convulsant is administered subcutaneously.

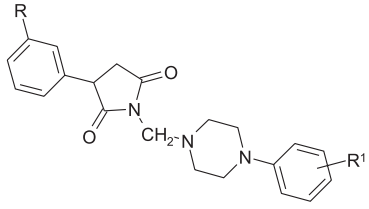
All the compounds were injected intraperitoneally into mice at the dose levels of 30, 100, and 300 mg/kg with anticonvulsant activity and neurotoxicity assessment at 0.5 and 4 hours after administration.

The neurological toxicity (NT) induced by a compound was detected in mice or rats using standardized rotorod test (19). Untreated control mice or rats, when placed on the rod, can maintain their equilibrium for a prolonged time period. The acute motor impairment can be demonstrated by the inability of animal to maintain equilibrium for a given time.

The results of preliminary screening for compounds **VIII**-**XXV** are presented in Tables 5 and 6.

According to the ASP dispositions compound **XVIII** was administrated orally into rats at a fixed dose of 30 mg/kg (MES test). This screen discloses the time of onset, the approximate time of peak effect (TPE) and the duration of anticonvulsant activity. At the same doses, the motor impairment was studied in parallel. Rats were tested at five time

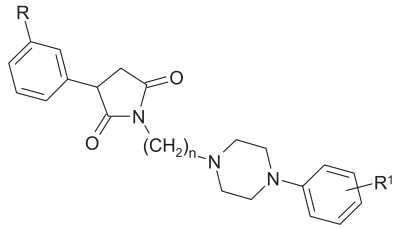
Table 5. Anticonvulsant screening project (ASP), results in mice for compounds **VIII**-**XIX**.



Compound	R	R_1	Intraperitoneal injection in mice			
			MES[a]		NT[b]	
			0.5 h	4 h	0.5 h	4 h
VIII	H	H	-	-	100	300
IX	H	2-F	-	-	300[1]	-
X	H	3-Cl	-	-	300	-
XI	H	4-Cl	-	-	-	-
XII	H	3-CF_3	-	-	-	-
XIII	H	3-CH_3	-	-	30	-
XIV	CH_3	H	-	-	30	-
XV	CH_3	2-F	-	-	300	300
XVI*	CH_3	3-Cl	-	300	-	-
XVII	CH_3	4-Cl	-	100	-	300
XVIII	CH_3	3-CF_3	300	100	300[14]	-
XIX	CH_3	3-CH_3	-	-	100	-
Phenytoin[c]	-	-	30	30	100	100

[a)] Doses of 30, 100 and 300 mg/kg were administrated. The values in the table indicate the minimum dose (mg/kg), whereby bioactivity was demonstrated. The dash (-) indicates an absence of activity at maximum dose administrated. [b)] Toxicity screen: the minimum dose of compound whereby toxicity was exhibited. [c)] Reference drug, data from Ref. (21). Response comments: [1] death, [14] unable to grasp rotorod.
* Compounds **XVI** revealed anti-MES activity at a dose of 100 mg/kg at 6 h

Table 6. Anticonvulsant screening project (ASP), results in mice for compounds **XX**-**XXV**.



Compound	R	R_1	n	Intraperitoneal injection in mice			
				MES[a]		NT[b]	
				0.5 h	4 h	0.5 h	4 h
XX	H	3-CF_3	2	100	100	300	300
XXI	H	H	3	-	100	100[1]	-
XXII	CH_3	H	2	-	100	-	-
XXIII	CH_3	2-F	2	-	-	-	-
XXIV	CH_3	3-Cl	2	-	-	-	-
XXV*	CH_3	3-Cl	3	300	100 30[1]		
Phenytoin[c]				30	30	1001	

For [a), b), c)] and Response comments see Table 5. *Compound **XXV** revealed anti-MES activity at a dose of 100 mg/kg at 6 h

Table 7. Anticonvulsant activity (MES test) of compound **XVIII** administrated orally to rats.

Compound	Oral administration to rats[a]				
	0.25 h	0.5 h	1 h	2 h	4 h
XVIII	0	1	2	1	0
Phenytoin[b]	1	4	3	3	3

[a] The data in the oral MES screen indicate the number of rats of four that were protected at a dose of 30 mg/kg. [b] Reference drug, data from ref. (21)

periods ranging from one quarter to 4 h post substance administration. The results are shown in Table 7.

RESULTS

The anticonvulsant profile of compounds **VIII**-**XXV** was established in the maximal electroshock (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) tests, after intraperitoneal injection into mice at doses of 30, 100 and 300 mg/kg. An observation was carried out at two different time intervals, namely 0.5 h and 4 h. The acute neurological toxicity (NT) was determined in the minimal motor impairment-rotorod screen (NT).

The MES and *sc*PTZ tests are claimed to detect compounds affording protection against generalized tonic-clonic seizures and generalized absence seizures, respectively. Thus they are recognized as the "gold standards" on the early stages of testing of new anticonvulsants (20). As shown in Tables 5 and 6, compounds **VIII**-**XXV** revealed protection only in the electrically induced seizures or were inactive. In this series, the most active were compounds **XVII**, **XVIII, XX**-**XXII** and **XXV**, which showed anti-MES activity at doses of 100 mg/kg both at 0.5 h and 4 h (**XX**) or only at 4 h (**XVII**, **XVIII**, **XXI** and **XXII**) after intraperitoneal injection in mice. Compounds **XVIII** and **XXV** revealed also protection at a dose of 300 mg/kg at 0.5 h. It is noteworthy that **XXV** active at a doses 100 mg/kg and 300 mg/kg was toxic at a dose of 30 mg/kg (mice were unable to grasp rotorod). The further results indicated that **XVI** was less active and provided protection at a dose of 300 mg/kg at 4 h. This compound was also effective at a dose of 100 mg/kg at 6 h. All the N-[(4-arylpiperazin-1-yl)-methyl]-3-phenyl-pyrrolidine-2,5-diones (**VIII**-**XIII**) as well as **XIV**, **XV**, **XIX**, **XXIII** and **XXIV** were devoid of activity.

The results from the rotorod neurotoxicity evaluations demonstrated that only five compounds, namely **XI**, **XII**, **XVI**, **XXIII** and **XIV,** did not cause neurotoxicity at the maximal dose administrated – 300 mg/kg. The other derivatives were found to be neurotoxic at a dose of 30 mg/kg (**XIII**, **XIV** and **XXV**), 100 mg/kg (**VIII**, **XIX**, **XXI**) or 300 mg/kg (**IX**, **X**, **XV**, **XVII**, **XVIII** and **XX**).

Compound **XVIII** was examined for its anticonvulsant activity and neurotoxicity in rat oral screen at a dose of 30 mg/kg in MES test (Table 7). This molecule revealed 50% protection at time point 1 h and 25% protection at 0.5 h. When given orally, **XVIII** was not neurotoxic. This compound was less active than phenytoin, used as reference substance for anticonvulsants effective in the electrically induced seizures.

In conclusion, among compounds studied the most active was N-[{4-(3-trifluoromethylphenyl)-piperazin-1-yl}-methyl]-3-(3-methylphenyl)-pyrrolidine-2,5-dione [**XVIII**], obtained as close analogue of model compound **III**. In the investigated series of compounds, the anticonvulsant activity depended on the presence of methyl group at the 3-phenyl moiety, the kind of substituents at the 4-arylpiperazine fragments, as well as the length of alkyl spacer between the imide and piperazine nitrogen atoms. In relation to Mannich bases **VIII**-**XIX**, the most active were 3-methylphenyl-pyrrolidine-2,5-diones containing the electron-withdrawing groups at the 4-arylpiperazine, in general. Interestingly, the removal of the 3-methyl group yielded inactive analogues. On the other hand, the elongation of the alkyl chain between the imide and piperazine nitrogen atoms from the methylene to ethylene or propylene increased the activity among the 3-phenyl-pyrrolidine-2,5-diones, in contrast to the 3-methyl analogues.

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